ANCA-associated vasculitis : factors involved in disease induction, survival and long-term complications

Chapter 12

Summary and general discussion

Vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA), including Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), Churg-Strauss syndrome (CSS), predominantly affects small- and medium-sized blood vessels. These ANCA-related syndromes form a distinct subset with overlapping features within the spectrum of primary vasculitic diseases; the respiratory tract and kidney are organ systems frequently involved.
The ANCA-associated vasculitides can be distinguished in two ways. First, a distinction based on diagnosis; subcategories then include WG, MPA, CSS, and idiopathic necrotizing and crescentic glomerulonephritis (iNCGN). A drawback of this method is that a disease pattern first being diagnosed as MPA can evolve into WG several years after diagnosis. A second drawback is that a diagnosis of WG can almost never be excluded, as granulomas must be present in a biopsy for this diagnosis to be made, and they may not be present in a particular biopsy, even though the patient has WG.
A second distinction is based on ANCA specificities. Proteinase 3 (PR3) and myeloperoxidase (MPO) are the major autoantigens in ANCA-associated vasculitis (AAV), although other ANCA specificities occasionally are seen. However, patients with a biopsy-proven "ANCA-related" vasculitic syndrome may very well be ANCA negative, especially when the vasculitis is limited or locoregional (for instance, patients with vasculitis of the upper respiratory tract with granulomatous inflammation in a nasal biopsy). Especially patients with CSS are often ANCA-negative (up to 70% of the cases).
As an introduction to the diseases studied in the subsequent chapters, *Chapter 1* gives a general overview of the ANCA-associated vasculitides and the current role of ANCA in the diagnosis and prognosis of patients with this disease.

Several drugs have been associated with the onset of AAV, including antirheumatic and antithyroid drugs. In *Chapter 2* we evaluate the potential of the antirheumatic drugs minocycline, sulfasalazine and penicillamine to induce ANCA. We tested 248 patients who had participated in clinical trials, providing us with pre- and post-exposure blood samples. From our study results, there was no suggestion of ANCA seroconversion due to these drugs.

Chapter 3 describes a related study in which we investigated the presence of ANCA after the use of antithyroid drugs. We hypothesized that ANCA and AAV may not only be induced during treatment with antithyroid drugs, but can also become manifest when medication has been ceased, possibly after years. To test this hypothesis, we evaluated patients with hyperthyroidism, on various antithyroid treatment modalities, for the presence of ANCA. We found that of 209 patients with hyperthyroidism, 12 patients (6%) were positive for myeloperoxidase- (MPO-), proteinase 3- (PR3-) and/or human leukocyte elastase- (HLE-) ANCA, and that the presence of ANCA was highly associated with (previous) treatment with antithyroid drugs.

Human Heat Shock Protein 60 (hHSP60) has been implicated in autoimmunity through molecular mimicry, based on the high degree of homology with HSP65 of micro-organisms leading to autoimmune recognition of the human protein. Additionally, sequence homology between hHSP60 and myeloperoxidase (MPO) has been described. MPO is a major autoantigen in vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA). In *Chapter 4*, we hypothesized that infections may trigger the ANCA response against MPO through hHSP60.
In 86 consecutive patients with ANCA-associated vasculitis (AAV), anti-hHSP60 and anti-mycobacterial HSP65 were measured by ELISA. Patients were compared with 69 healthy controls (HC). We found that antibodies against mHSP65 are higher in AAV compared to HC, and anti-hHSP60 antibodies are higher in patients with MPO-ANCA than in patients with PR3-ANCA and HC. Although this finding may be indicative for cross-reactivity between MPO-ANCA and hHSP60, additional assays did not support our hypothesis.

In *Chapter 5* we investigated whether genetic factors, regulating T cell activation, are involved in the onset of AAV. T cell activation is regulated by inhibitory molecules such as PD-1 and CTLA-4, whose expression in turn may be affected by gene polymorphisms. We investigated the relationship between polymorphisms in *PDCD1* and *CTLA4* and the presence of AAV, and found that AAV is associated with the presence of a G allele at the +49 position of *CTLA4*; this association is even stronger if also the PD-1.5 T allele is absent in the genome.

This led us to hypothesize that for autoimmune disease, a mutation in more than one inhibitory receptor predisposes to the development of disease, for instance, AAV. Furthermore, disease subsets are associated with different genetic polymorphisms in *CTLA4*.

Chapter 6 presents a long-term follow-up study in patients with PR3-ANCA associated vasculitis with renal involvement. Eighty-five patients that were diagnosed between 1982 and 1996 were followed for at least 5 years, to investigate prognostic factors for patient and renal survival. We discovered that early death and failure to recover renal function in PR3-ANCA associated vasculitis is associated with age above 65 years and dialysis dependency at diagnosis. Furthermore, long-term renal survival is determined by renal relapses during follow-up only. Slow, progressive renal failure without relapses, not infrequently found in patients with MPO-ANCA associated vasculitis, is rarely observed in patients with PR3-ANCA associated vasculitis with glomerulonephritis.

In patients with PR3-ANCA, renal function is stable during remission, but declines with every renal relapse. In patients with MPO-ANCA a slowly progressive course with patients developing dialysis dependency during follow-up without signs of clinically active disease is often observed. In *Chapter 7* we studied the clinical and histological differences at diagnosis between patients with PR3-ANCA and patients with MPO-ANCA, and we investigated whether these differences explain the distinctive outcomes in AAV. Eighty patients (46 with MPO- and 34 with PR3-ANCA) were included. Renal lesions differed between patients with PR3- and MPO-ANCA, being more acute in PR3-ANCA, and more chronic in MPO-ANCA. However, despite the overt differences in renal biopsies, ANCA specificity was not a risk factor for patient death or the development of renal failure during long-term follow-up. Risk factors for death were female gender, age 65 years or higher at diagnosis and serum creatinine level at biopsy. The development of renal failure during follow-up was associated with female gender, a lower percentage of normal glomeruli, and the chronicity score in renal biopsy.

Next to patient and renal survival, we investigated factors that are associated with disease-free survival. In *Chapter 8*, we analyzed

disease-free survival in patients with AAV treated with cyclophosphamide only or switched to azathioprine after 3 months of full remission with cyclophosphamide. Included were 128 patients of whom 44 (34%) switched to azathioprine. In patients with PR3-ANCA associated vasculitis switched to azathioprine (n=33) a positive C-ANCA titer at the moment of treatment switch (n=13) was significantly associated with the occurrence of relapse. In patients with a negative ANCA titer at the time of switch to azathioprine (n=20), disease-free survival at 2 and 4 years was 80% and 62%, which was identical to patients treated with cyclophosphamide only. In patients who were ANCA positive at the time of treatment switch disease-free survival at 2 and 4 years was only 58% and 17%. Thus, switching cyclophosphamide to azathioprine after induction of remission in patients with PR3-ANCA associated vasculitis who are still ANCA positive at the time of treatment switch is associated with a high risk of developing relapses.

A serological marker for the extent and progression of atherosclerosis, anti-oxidized low-density lipoprotein (oxLDL), is studied in AAV in *Chapter 9*. Three methods for detection of anti-oxLDL are compared between patients with MPO-ANCA and patients with PR3-ANCA. We measured antibody levels to LDL after treatment with hypochlorite, MDA or copper in sera of ANCA-positive vasculitis patients, and compared them to antibody levels in sera of hemodialysis patients and healthy controls. We found that anti-oxLDL antibodies are enhanced in AAV patients (MDA-LDL and hypochlorite-LDL) and in dialysis patients (hypochlorite-LDL), when compared to controls. Furthermore, patients with MPO-ANCA associated vasculitis had higher levels of antibodies to hypochlorite-LDL than patients with PR3-ANCA associated vasculitis. We concluded that our newly developed assay to measure antibodies to ox-LDL, in which hypochlorite-LDL is used as substrate, seems a more sensitive assay than traditional assays to measure these antibodies. Furthermore, our results suggest that enhanced MPO-mediated LDL oxidation in patients with MPO-ANCA reflects the idea that circulating MPO-ANCA prevents inactivation of circulating MPO activity by ceruloplasmin in these patients.

In *Chapter 10*, we analyzed the number of circulating $CD4^+CD28^-$ T

cells in relation to the presence of atherosclerotic damage in patients with AAV. Furthermore, we determined whether antibodies against cytomegalovirus (CMV) and human Heat Shock Protein 60 (hHSP60) were related to the presence of $CD4^+CD28^{null}$ T cells in AAV. Forty patients with inactive AAV were included in this study. Patients' spouses were recruited as healthy controls (HC, n=38). Intima-media thickness (IMT) and pulse wave velocity (PWV) were measured. Our results showed that there is evidence of atherosclerotic damage in patients with AAV, as measured by PWV, although carotid plaque size did not seem to differ. $CD4^+CD28^{null}$ T cells are relatively increased in AAV and related to previous CMV infection. However, there was no correlation between $CD4^+CD28^{null}$ T cells and increased IMT or PWV.

Finally, in *Chapter 11*, we summarize the current perceptions on involvement of the immune system in atherosclerosis, with particular attention to possible immunological causes of accelerated atherosclerosis in AAV.

General conclusions

The work described in this thesis is focused on ANCA-associated vasculitis. Its subjects are factors involved in disease onset (*Chapters 2, 3, 4, 5*), factors involved in patient and renal survival (*Chapters 6, 7*) and disease-free survival (*Chapter 8*), and the presence and development of atherosclerosis at diagnosis and during follow-up of AAV (*Chapters 9, 10*). Although we are still a long way from curing this disease, some issues have become clear.

First of all, if the factors involved in disease onset are known, therapies or strategies may evolve that might be able to prevent vasculitis, or cure this disease at an early stage. We studied several drugs that have been associated with the induction of ANCA, and possibly of AAV. A relationship between the use of antithyroid drugs and the presence of AAV was established (*Chapter 3*); on the other hand, a connection between certain antirheumatic drugs and vasculitis is still questionable (*Chapter 2*).

Although medication use has sometimes been associated with vasculitis, infections (and especially infection with *Staphylococcus aureus*) have often been linked to vasculitis. Antibodies against bacterial and human Heat Shock Proteins (HSPs) are elevated in AAV,

especially in MPO-AAV (*Chapter 4*). Nevertheless, we could not establish a correlation between the presence of antibodies against HSPs and the presence of ANCA.

Next to the possible induction of ANCA by infection or medication use, we studied a possible cause for the involvement of T cells in the onset of AAV. We found that certain gene polymorphisms that lead to a reduced expression or function of the T cell inhibitory molecule CTLA-4, in combination with a gene polymorphism in a second inhibitory molecule (PD-1), are associated with the presence of AAV (*Chapter 5*). This may indicate that reduced inhibition of T cells influences the occurrence of AAV.

Second, we investigated whether we could identify factors that are involved in patient, renal and disease-free survival, possibly leading to a more tailored approach of patients with AAV. We observed that age and reduced renal function are primarily associated with abridged short-term patient and renal survival (*Chapter 6, 7*), whereas long-term renal survival is strongly dependent on the occurrence of renal relapses (*Chapter 6*). In our long-term survival analysis, we found that atherosclerosis and malignancy are the most common causes of death in patients with AAV (*Chapter 6*). Among other reasons, the high chance of malignancy with the use of the standard therapy against AAV (cyclophosphamide and prednisolone), has led investigators to propose a less aggressive treatment for remission maintenance, giving patients azathioprine instead of cyclophosphamide. The use of azathioprine, however, may not be as successful as cyclophosphamide in preventing the occurrence of relapses of disease activity during follow-up. In patients that remain positive for ANCA, the use of azathioprine may lead to a strongly reduced disease-free survival (*Chapter 8*).

Third, we investigated the presence of atherosclerosis as a consequence of ANCA-associated vasculitis, at diagnosis and during follow-up. One serological marker of atherosclerosis, antibodies against oxidized LDL, was found to be already present at diagnosis of AAV, particularly in patients with MPO-ANCA (*Chapter 9*). During long-term follow-up, we also found evidence for the presence of accelerated atherosclerosis in patients with AAV (*Chapter 10*). Furthermore, immunological abnormalities were found in patients with AAV that are also common in patients with atherosclerosis (*Chapter 10*).

Future perspectives

Pathophysiology of ANCA-associated vasculitis

In the last years, more insight has been gained regarding the pathophysiological role of ANCA in ANCA-associated vasculitis, leading some investigators to propose the term "ANCA vasculitis" instead[1]. These investigators have demonstrated in a mouse model that ANCA alone are sufficient for disease induction[1]. The MPO knockout mice they used were immunized with murine MPO, leading to the development of MPO-ANCA in these mice. Injecting these antibodies in mice with MPO led to the development of vasculitis and glomerulonephritis. Of course, the question still remains how a person can suddenly develop an autoimmune response against MPO or, for that matter, PR3. Many theories have been developed, with molecular mimicry between bacterial and human proteins as a favored one; one that has also been studied by us (*Chapter 4*). Recently, a second theory has gained much attention after proving its pathogenecity in a mouse model. Pendergraft and colleagues described an autoimmune response induced by the presence of a peptide that is antisense or complementary to PR3[2]. After immunizing mice with antisense PR3, they found an autoimmune reaction to blood vessels in these mice. The mice had not only developed antibodies against antisense PR3, but also to the sense peptide PR3. Furthermore, antibodies in sera from patients with Wegener's granulomatosis reacted not only with PR3, but also with antisense human PR3. An explanation for this remarkable finding is that when the sense and antisense molecules of a peptide are complementary to each other, an immune response against the antisense peptide may induce anti-idiotypic antibodies (autoantibodies) that cross-react with the autoantigen. The antisense immune response may, in turn, be a result of molecular mimicry with bacterial proteins – the antisense PR3 protein, for instance, shares sequence homology with a protein of *Staphylococcus aureus*[2], a pathogen that has been repeatedly linked to WG.

The role of T cells in the pathogenesis of AAV is still much less clear. Obviously, T cells are necessary for the production of IgG ANCA[3], and T cells are present in inflammatory lesions in AAV[4,5]. On the other hand, in the mouse model previously described, infusion of only T cells does not lead to inflammation in mice, in contrast to infusion of only MPO-ANCA. Nevertheless, infusion of both T cells and MPO-ANCA leads to

much more inflammation in the mouse model than infusion of MPO-ANCA alone[1].

Since the T cell response is necessary for the production of ANCA, it is interesting to investigate why a T cell would react with autoantigens. We have investigated one hypothesis for hyperreactivity of T cells; impairment of counterregulation due to polymorphisms in genes coding for the immunoregulatory molecules CTLA-4 and PD-1 (*Chapter 5*). Of course, these molecules are not the only ones involved in the regulation of the immune response. Besides, since these gene polymorphisms are also found in many healthy persons, and various other autoimmune diseases, it seems that more than a gene polymorphism is necessary for the development of AAV. It could be interesting to look whether a combination of factors that are thought to be at least partially responsible for the induction of AAV coincide in patients with AAV. For instance, are patients with immunoregulatory polymorphisms more susceptible to drug-induced vasculitis?

Treatment and follow-up of ANCA-associated vasculitis

Many studies have described potential prognostic factors for patient and renal survival (summarized in *Chapters 6* and *7*). Unfortunately, most of these prognostic factors can not readily be influenced by therapy. An exception to this is renal failure at diagnosis, defined as serum creatinine above 500 µmol/L or dialysis dependency. Renal failure at diagnosis is highly associated with early death and failure to recover renal function (*Chapters 6* and *7*). A recent study has shown that plasma exchange may be beneficial to these patients, drastically improving their survival without need for renal replacement therapy within one year[6].

Induction therapy with cyclophosphamide and prednisolone is still the golden standard for generalized ANCA-associated vasculitis. Long-term use of cyclophosphamide, however, leads to serious morbidity, even mortality, mostly due to malignancies several years after diagnosis[7]. To decrease the chance on developing these long-term side effects, other drugs have been tested for remission maintenance and remission induction. Azathioprine at first seemed equally able to suppress disease activity[8]; however, during long term observation, we showed that azathioprine use does lead to more relapses of disease activity, especially when patients were C-ANCA positive at the time of treatment

switch from cyclophosphamide to azathioprine (*Chapter 8*). Two trials are currently on their way; one testing the effect of longer use of azathioprine in patients that remain C-ANCA positive; the other testing mycophenolate mofetil (MMF) as treatment for remission maintenance. On the other hand, perhaps induction should be made more aggressive and aiming at an absent ANCA titer when treatment switch is considered. Adding anti-CD20 or anti-TNFa to induction treatment can be interesting in this perspective.

Atherosclerosis and ANCA-associated vasculitis

Since the introduction of cyclophosphamide and prednisolone as standard treatment, AAV has evolved from an almost uniformly fatal disease to a chronic disorder. Long-term follow-up, however, has shown that late consequences of the disease and / or its treatment can result in serious morbidity. Cardiovascular events occur in a substantial percentage of patients with AAV[9], leading us to investigate the presence of accelerated atherosclerosis in these patients. Although we did not find marked differences between patients and healthy, age-matched controls, subtle distinctions were there. It would be very interesting to know how these differences progress.

Of course, the methods we used to detect atherosclerosis in AAV patients may not have been sensitive enough for us to detect large differences between patients and controls. With a recent method described by Kooi et al[10] it would be possible to specifically look at rupture-prone atherosclerotic plaques, which may more adequately assess the increased risk of cardiovascular events for patients with AAV than intima-media thickness measurements or pulse-wave velocity. Assessment of myocardial perfusion could also be a way to detect atherosclerotic lesions[11].

Since the presence of $CD4^+CD28^{null}$ T cells was clearly related to myocardial infarction in the studies by Liuzzo et al[12,13], one point for follow-up could be the occurrence of cardiovascular events, though the number of patients we studied is small. Animal studies could perhaps give us more insight in the role of $CD4^+CD28^{null}$ T cells in atherosclerosis. Furthermore, the natural course of atherosclerosis in vasculitis could be studied in the mouse model for AAV.

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Samenvatting

Vasculitis geassocieerd met antineutrofiele cytoplasmatische antistoffen (ANCA), zoals Wegener's granulomatose, microscopische polyangiitis (MPA), en het syndroom van Churg-Strauss (CSS), komt met name voor in de kleine en middelgrote bloedvaten. Deze ANCA-gerelateerde aandoeningen vormen een speciale categorie met overlappende symptomen binnen het spectrum van de vasculitiden van de kleine vaten; de luchtwegen en de nieren zijn daarbij vaak aangedaan.
Er kan op 2 manieren onderscheid gemaakt worden binnen de ANCA-geassocieerde vasculitiden. De eerste manier is gebaseerd op de verschillende diagnoses; subcategorieën zijn dan WG, MPA, CSS en idiopathische necrotiserende en crescentische glomerulonefritis (iNCGN). Een nadeel van deze methode is dat een syndroom dat eerst gediagnosticeerd wordt als MPA, jaren na de daignose kan evoluëren naar een WG. Een tweede nadeel is dat de diagnose WG bijna nooit geëxcludeerd kan worden, aangezien de aanwezigheid van granulomen in minimaal een biopt voorwaarde is voor deze diagnose. Deze kunnen echter bij het maken van het biopt gemist worden, waardoor de diagnose WG niet gesteld kan worden zelfs als deze wel de onderliggende oorzaak is.
Een tweede onderscheid tussen de aandoeningen is gebaseerd op ANCA specificiteiten. Proteinase 3 (PR3) en myeloperoxidase (MPO) zijn veruit de belangrijkste autoantigenen in ANCA-geassocieerde vasculitis (AAV), hoewel sporadisch andere specificiteiten gevonden worden. Echter, patiënten met een biopt bewezen "ANCA-gerelateerd" syndroom kunnen ANCA negatief zijn, vooral wanneer de ziekte gelimiteerd of locoregionaal is (bijvoorbeeld patiënten met vasculitis van de bovenste luchtwegen en granulomateuse ontsteking in een neusbiopt). Met name patiënten met CSS zijn vaak ANCA negatief (tot 70% van de patiënten).
Hoofdstuk 1 geeft als introductie tot het ziektebeeld een overzicht van de ANCA-geassocieerde vasculitiden en de huidige rol van ANCA in de diagnose en prognose van patiënten met deze ziekte.

Verscheidene medicamenten zijn geassocieerd met het ontstaan van AAV, onder andere anti-reumatische medicijnen en thyreostatica. In *Hoofdstuk 2* onderzoeken we of de anti-reumatische medicijnen minocycline, sulfasalazine en penicillamine ANCA kunnen induceren. We testten hiervoor 248 patiënten die hadden geparticipeerd in clinical

trials, waardoor wij serum van voor en na de blootstelling tot onze beschikking hadden. Onze resultaten lieten geen aanwijzingen zien voor het ontstaan van ANCA door het gebruik van deze medicijnen.

Hoofdstuk 3 beschrijft een gerelateerde studie waarin wij de aanwezigheid van ANCA na het gebruik van thyreostatica onderzochten. We hypothetiseerden dat ANCA niet alleen kan ontstaan tijdens het gebruik van thyreostatica, maar ook jaren later, wanneer de thyreostatica gestopt zijn. Om deze hypothese te testen onderzochten wij patiënten met hyperthyreoïdie, met verschillende vormen van behandeling, op de aanwezigheid van ANCA. Van de 209 patiënten met hyperthyreoïdie hadden 12 patiënten (6%) MPO-, PR3- of human leukocyte elastase (HLE)-ANCA; de aanwezigheid van ANCA was sterk geassocieerd met thyreostatische behandeling.

Humaan heat shock protein 60 (hHSP60) wordt een rol toegedicht bij het ontstaan van autoimmuniteit door molecular mimicry, veroorzaakt door een sterke overeenkomst met HSP65 dat gevonden wordt bij micro-organismen. Daarnaast is overeenkomst tussen hHSP60 en MPO beschreven. MPO is een van de belangrijkste autoantigenen in ANCA-geassocieerde vasculitis. In *Hoofdstuk 4* hypothetiseerden we dat infecties het ontstaan van ANCA gericht tegen MPO kunnen induceren via kruisreactiviteit met hHSP60. In 86 patiënten met AAV werden antistoffen tegen hHSP60 en mycobacterieel HSP65 gemeten met behulp van ELISAs. Deze patiënten werden vergeleken met 69 gezonde controles (HC). We zagen dat antistoffen tegen mHSP65 hoger waren in AAV patiënten in vergelijking met HC, en anti-hHSP60 antistoffen waren hoger in patiënten met MPO-ANCA dan in patiënten met PR3-ANCA en HC. Dit zou kunnen wijzen op kruisreactiviteit, maar in aanvullende onderzoeken bleef bewijs van kruisreactiviteit uit.

In *Hoofdstuk 5* onderzochten we of genetische factoren, van invloed op de T-cel regulatie, betrokken zijn bij het ontstaan van AAV. T-cel activatie wordt gereguleerd door inhibitoire moleculen zoals PD-1 en CTLA-4. De expressie van deze moleculen wordt beïnvloedt door genetische polymorfismen. We onderzochten de relatie tussen polymorfismen in *PDCD1* en *CTLA4* en de aanwezigheid van AAV, en we zagen dat AAV geassocieerd is met de aanwezigheid van het G allel op

de positie +49 van *CTLA4*; deze relatie wordt sterker als ook het PD-1.5 T allel niet aanwezig is in het genoom. Dit resultaat leidde naar onze huidige hypothese; voor het ontstaan autoimmuunziekten zoals AAV is waarschijnlijk meer dan één mutatie nodig in inhibitoire receptoren. Daarnaast zijn verschillende subgroepen binnen AAV geassocieerd met verschillende *CTLA4* polymorfismen.

Hoofdstuk 6 presenteert een lange termijn follow-up studie van patiënten met PR3-ANCA geassocieerde vasculitis met nierbetrokkenheid. Vijfentachtig patiënten, gediagnosticeerd tussen 1982 en 1996 werden minimaal 5 jaar gevolgd om te onderzoeken welke factoren van invloed zijn op overleving van patiënten en nierfunctie. We zagen dat snel overlijden en blijvend verlies van nierfunctie in PR3-ANCA vasculitis geassocieerd is met leeftijd boven 65 jaar en dialyse-afhankelijkheid ten tijde van diagnose. Verder vonden wij dat alleen het optreden van renale recidieven voorspellend is voor overleving van de nierfunctie op lange termijn. Langzame achteruitgang van de nierfunctie zonder activiteit van de vasculitis, zoals regelmatig gezien wordt bij MPO-ANCA vasculitis, komt zelden voor bij PR3-ANCA vasculitis.

In patiënten met PR3-ANCA is de nierfunctie stabiel tijdens remissie, maar gaat achteruit met elk renaal recidief. In patiënten met MPO-ANCA wordt juist vaak gezien dat de nierfunctie versneld achteruit gaat, zonder dat er activiteit van de ziekte te herkennen is. In *Hoofdstuk 7* bestudeerden we welke klinische en histologische verschillen aanwezig zijn tussen patiënten met PR3-ANCA en patiënten met MPO-ANCA, en we onderzochten of deze verschillen de verschillen in outcome kunnen verklaren. Tachtig patiënten (46 met MPO-ANCA en 34 met PR3-ANCA) werden geïncludeerd in de studie. De afwijkingen in het nierbiopt verschilden tussen patiënten met PR3- en MPO-ANCA; in PR3-ANCA zijn de laesies meer acuut, terwijl in MPO-ANCA meer chronische schade wordt gezien. Desondanks was ANCA specificiteit geen risicofactor voor overlijden van de patiënt of nierfalen tijdens follow-up. De risicofactoren voor overlijden die wij wel zagen waren vrouwelijk geslacht, leeftijd boven 65 jaar, en serum creatinine ten tijde van het biopt. Het ontstaan van nierfalen was geassocieerd met het vrouwelijk geslacht, een lager percentage van normale glomeruli,

en de chroniciteitsscore van het nierbiopt.

Naast patiënten- en renale overleving hebben we ook factoren onderzocht die van invloed kunnen zijn op ziektevrije overleving. In *Hoofdstuk 8* analyseerden we ziektevrije overleving in patiënten met AAV die ofwel met cyclofosfamide werden behandeld, danwel na 3 maanden volledige remissie van cyclofosfamide naar azathioprine overschakelden. Er werden 128 patiënten geïncludeerd; 44 hiervan (34%) gingen over op azathioprine. In patiënten met PR3-ANCA (n=33), die een positieve ANCA titer hadden op het moment van swith (n=13), was de kans op een recidief significant hoger. In patiënten met een negatieve ANCA titer ten tijde van switch (n=20) was de ziektevrije overleving op 2 en 4 jaar respectievelijk 80% en 62%; dit was gelijk aan de ziektevrije overleving van patiënten die cyclofosfamide bleven gebruiken. In patiënten die ANCA positief waren, was de ziektevrije overleving op 2 en 4 jaar slechts 58% en 17%. Wij concludeerden dan ook dat de overstap van cyclofosfamide naar azathioprine bij patiënten met PR3-ANCA geassocieerde vasculitis, die ANCA positief zijn ten tijde van de overstap, het risico op recidieven sterk verhoogd is.

Een serologische marker voor de uitgebreidheid en progressie van atherosclerose, antilichamen tegen geoxideerd low-density lipoprotein (oxLDL), werd bestudeerd in AAV in *Hoofdstuk 9*. Drie manieren om anti-oxLDL te detecteren werden vergeleken tussen patiënten met MPO-ANCA en patiënten met PR3-ANCA. We bepaalden de hoeveelheid antilichamen tegen LDL, nadat dit behandeld werd met hypochloriet, MDA of koper, in ANCA-positieve patiënten, en vergeleken deze met de hoeveelheid antilichamen in sera van hemodialysepatiënten en gezonde controles. We zagen dat de hoeveelheid anti-oxLDL antilichamen waren toegenomen in AAV patiënten (MDA-LDL en hypochloriet-LDL) en in dialysepatiënten (hypochloriet-LDL) in vergelijking met gezonde controles. Daarnaast zagen we dat patiënten met MPO-ANCA meer antilichamen tegen hypochloriet-LDL hadden dan patiënten met PR3-ANCA. We concludeerden dat onze nieuw ontwikkelde test voor het meten van anti-oxLDL, met hypochloriet-LDL als substraat, gevoeliger is voor het detecteren van deze antilichamen dan traditionele testen. Daarnaast lijkt het zo te zijn dat oxidatie van LDL met behulp van MPO

in patiënten met MPO-ANCA voorkomt en veroorzaakt wordt door de circulerende MPO-ANCA antilichamen, die door hun binding met MPO de binding van MPO aan zijn natuurlijke remmer, ceruloplasmine, voorkomen.

In *Hoofdstuk 10* analyseerden we de hoeveelheid circulerende $CD4^+CD28^-$ T cellen in relatie tot de aanwezigheid van atherosclerotische schade in patiënten met AAV. Daarnaast bepaalden we of antilichamen tegen cytomegalovirus (CMV) en humaan heat shock protein 60 (hHSP60) geassocieerd waren met de aanwezigheid van $CD4^+CD28^{null}$ T cellen in AAV. Veertig patiënten in remissie werden in de studie geïncludeerd. De echtgenoten van de patiënten werden als gezonde controles (HC) gevraagd (n=38). De intima-media dikte (IMT) en pulse-wave velocity (PWV) werden bepaald. We zagen dat er bewijs voor atherosclerotische schade is in patiënten met AAV, zoals gemeten met de PWV; aan de andere kant werd er geen verschil in IMT gevonden. $CD4^+CD28^{null}$ T cellen zijn relatief verhoogd in AAV en geassocieerd met vroegere CMV infectie. We zagen geen relatie tussen $CD4^+CD28^{null}$ T cellen en verhoogde IMT of PWV.

Tot slot worden in *Hoofdstuk 11* de huidige opvattingen van de betrokkenheid van het immuunsysteem in atherosclerose samengevat, met speciale aandacht voor mogelijke immunologische oorzaken van versnelde atherosclerose in AAV.